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OCCURRENCE OF *BACILLUS CEREUS* IN SOME MILK-BASED DESSERTS

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ABSTRACT

A total of one hundred and fifty milk-based desserts samples including rice with milk, pudding, and custard (50 each) were collected from different localities in Assiut city, Egypt. The collected samples were examined for the isolation and enumeration of *Bacillus cereus*. The Most Probable Number technique was used for the enumeration of *Bacillus cereus* using tryptone soy polymyxin broth and Mannitol Egg Yolk Polymyxin (MYP) agar. The incidence of *Bacillus cereus* was 62%, 44% and 32% in the examined rice with milk, pudding, and custard samples, respectively. The *Bacillus cereus* count in most of the examined samples was in the range of 10-<10^2 CFU/g. The public health importance of *Bacillus cereus* was also discussed.

Key words: *Bacillus cereus*, rice with milk, pudding, custard.

INTRODUCTION

Bacillus cereus is a Gram-positive, aerobic spore-forming bacilli, commonly present in various natural environments and food matrices (Fiedoruk *et al.*, 2017). *B. cereus* is responsible for causing diarrhea, emesis, fatal meningitis, and spoilage of different food products (Evreux *et al*., 2007).

Bacillus cereus food poisoning has two forms; the diarrheal form which is caused by ingesting large numbers of bacterial cells or their spores in a contaminated food, and the emetic form caused by ingesting food contaminated with the preformed toxin. The diarrheal type is primarily manifested by abdominal cramps and diarrhea following an 8 to 16 hrs incubation period (Murray *et al.*, 2007). The emetic form is commonly caused by a toxin (cereulide), which is heat stable causing nausea and vomiting 1–5 hrs after consumption (Hoton *et al.*, 2005). The similarities between the symptoms of the emetic disease and *Staphylococcus aureus* intoxication and the similarities between the diarrheal disease and that caused by *Clostridium perfringens* type A food poisoning create confusion to distinguish *B. cereus* food poisoning (Stenfors *et al*., 2008).

While there are various estimates of the number of *B. cereus* cells required to cause illness, there is a general agreement that foods containing <10^3 CFU/g are safe for human consumption (Vilas-Boas *et al.*, 2007). Meanwhile, it is reported that *B. cereus* should be 10^5–10^8 CFU/g in order to cause emetic or diarrheal illness (EFSA, 2005). Although, a small dose (<10^3 CFU/g) may cause disease in susceptible individuals (Szabo *et al.*, 1984). *B. cereus* food poisoning results from consumption of contaminated foods, as well as improper handling, storage and cooling of cooked foodstuffs (Schneider *et al*., 2004). Under improper storage conditions of food after cooking, the spores germinate and the vegetative cells multiply (Logan, 2011).

Presence of *B. cereus* in the dairy products is not only of concern as a public health hazard but also as a cause of economic losses through the reduction of shelf life and the spoilage of the contaminated products. It produces spoilage enzymes such as proteases, lipases, and lecithinases (Fagerlund *et al*., 2004), it causes bitter-rotten off-flavors due to the protease activity and fruity-rancid off-flavors due to the lipolytic activity (Stenfors *et al*., 2008).

B. cereus forms resistant spores that spread easily; therefore there is a risk in its transmission through processed, pasteurized, sterilized, and heat-treated food products (Kotiranta *et al*. 2000). Specific food types tend to be associated with illnesses caused by *B. cereus*. Desserts and dairy products are most frequently the vehicles for the transmission of the diarrheal form of the illness, whereas rice is the main vehicle of the emetic illness. The emergence of psychrotrophic and thermophilic species may mean that *B. cereus* will be of increasing concern to the food industry in the future (Griffiths and Schraft, 2017). It is believed that growth of psychrotrophic strains to high numbers in refrigerators is more significant than toxin production at low temperatures. Moreover, the psychrotrophic strains often limit the

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keeping quality of pasteurized milk and its products (Adams and Moss, 2008).

Because of the ubiquitous distribution of *B. cereus* in the environment, the prevention of food contamination with the organism or its spores is almost impossible. However, high numbers of *B. cereus* are needed in the food for the food-borne disease to occur, this is frequently associated with holding food under conditions allowing active growth of the organism; it could be through poor refrigeration, slow or inadequate cooling, or holding food warm below 60°C. Therfore, control measures should be directed to prevent spore germination and to prevent the growth of vegetative cells, which might be the approach to effectively prevent and control the spread of this pathogen. Thorough cooking of foods will likely destroy the vegetative cells and spores, however, heat treatment below 100°C might not be effective against spores. Also, non-refrigerated storage of food should be avoided and it should be rapidly and efficiently cooled to less than 7°C (Griffiths and Schraft, 2017).

Since *B. cereus* has the ability to grow in wide varieties of food, capable of forming heat-resistant spores, and able to produce toxins adapting heat treatment and cold storage, thus posing risks to the consumers. Additionally, cases of *B. cereus* food poisoning through milk-based desserts have been reported by Johnson (1984); Meer *et al.* (1991); Andersson *et al.* (1995); Schoeni and Wang (2005). Because the dairy desserts are widely consumed in Egypt, this study was conducted to determine the occurrence of *B. cereus* and to evaluate its load in some milk-based desserts sold in Assiut city markets.

MATERIALS AND METHODS

I. Sampling

A total of 150 milk-based desserts samples were collected from different dairies and pastry shops in Assiut city, Egypt. The samples were rice with milk, pudding (Mahallabia in Arabic dialect), and custard (50 each). Each sample was obtained as sold to the public and dispatched directly to the laboratory with a minimum of delay. The preparation and handling of the samples were done according to APHA (1992).

II. Isolation of *B. cereus*

An appropriate amount of each prepared sample was inoculated into brain heart infusion broth tubes, then was incubated for 24 h at 30°C. An aliquot from the incubated tubes was streaked onto a plate of MYP agar and incubated at 30°C for 24 h. Pink, lecithinase-positive colonies were transferred to nutrient agar slants and incubated at 30°C for 24 h for further identification.

III. Enumeration of *B. cereus*

Enumeration of *B. cereus* was done using the Most Probable Number technique (MPN) according to Tallent *et al.* (2012). Eleven grams of the prepared sample was aseptically transferred to 99 ml of sterile 0.l% peptone water and then thoroughly mixed to be emulsified completely to obtain a dilution 1/10, from which decimal dilutions were prepared. One ml from the previously prepared dilutions was added to tryptone soy polymyxin broth tubes (3 tubes for each dilution). The inoculated tubes were incubated at 30°C for 48 h. Loopfulls from the positive tubes were streaked onto Mannitol Egg Yolk Polymyxin (MYP) agar plates and incubated at 30°C for 24 h. The MPN of *B. cereus*/g of sample was obtained using the MPN table (Peeler *et al*., 1992).

IV- Identification of the isolates

The isolated organisms were identified microscopically and biochemically according to (Procop *et al*., 2017). The biochemical tests used for the identification were motility, catalase, nitrate reduction, citrate utilization, gelatin hydrolysis and indole tests. The identified *B. cereus* strains were large Gram-positive rods with non-swelling spore, produce lecithinase and do not ferment mannitol on MYP agar, reduce nitrate to nitrite and indole negative. They were motile, catalase positive, citrate utilization positive and gelatin hydrolysis positive.

RESULTS

Table 1: Incidence of *B. cereus* in the examined milk-based desserts samples.

The examined sampless	The No. of examined samples	The positive samples	
		No.	%
Rice with milk	50	31	62
Pudding	50	22	44
Custard	50	16	32

Table 2: Frequency distribution of the positive samples based on their *B. cereus* count.

Intervals CFU/g	Rice with milk		Pudding		Custard	
	No.	%	No.	%	No.	%
3-<10	-	-	3	13.64	3	18.75
$10-<10^2$	19	61.29	15	68.18	12	75
$10^2-<10^3$	10	32.26	3	13.64	1	6.25
$10^3-<10^4$	2	6.45	1	4.54	-	-
Total	31	100	22	100	16	100

Table 3: Microbial quality* of the examined samples based on their *B. cereus* count.

The examined samples	*B. cereus* CFU/g							
	Good		Acceptable		Unsatisfactory		Potentially hazardous	
	$<10^2$		$10^2-<10^3$		$10^3-<10^4$		$\geq10^4$	
	No./50	%	No./50	%	No./50	%	No./50	%
Rice with milk	38	76	10	20	2	4	-	-
Pudding	46	92	3	6	1	2	-	-
Custard	49	98	1	2	-	-	-	-

* According to the public health guidelines (NSW Food Authority, 2009)

DISCUSSION

Milk-based desserts are popular dairy food prepared from ingredients that milk is the basic constituent. They are palatable, nutritious and relatively inexpensive dairy food. These types of desserts are consumed in Egypt by a wide range of people of all ages and are usually served cooled (AL-Gendi, 2004). Rice with milk and pudding (Mahallabia) are the most widely consumed dairy desserts in Egypt (EL-Shaar, 1993). Milk-based products are good media for the growth of microorganisms because of their high nutritive value, almost neutral pH and long storage periods (Bell and Kyriakides, 1998). The bacteria most frequently found to contaminate milk-based desserts are members of the Enterobacteriaceae group, *Pseudomonas spp.*, and *Bacillus spp.* (in particular *B. cereus*) (Lewis and Dale, 1994).

Dairy-based foods are important means for the transmission of different pathogens especially in places where the hygienic measures are not strictly adopted (Meyer-Broseta *et al*., 2003). Assessing the exposure to *B. cereus* is an important issue in estimating the risk for food-borne disease by this microorganism. The actual disease symptoms, however, are caused by toxins, either produced during growth in the gut; enterotoxins or during growth in food; emetic toxin (Adams and Moss, 2008).

Dairy desserts in Egypt are locally produced by traditional retail shops and the production takes place manually in stores. Custard is made from highly nutritive raw materials; milk and sugar and is easily spoiled by the multiplication of specific microbial contaminants (Arakawa *et al*., 2008).

The Most probable number (MPN) technique was used for enumeration of *B. cereus* in this study as it is described in reference methods and is recommended for routine surveillance of products in which small numbers of *B. cereus* are expected (Bennett *et al*., 2015).

The results recorded in Table 1 revealed that *B. cereus* was detected in 62%, 44% and 32% of the examined rice with milk, pudding, and custard samples, respectively. Nearly similar results were recorded by Al- Ashmawy *et al*. (1996); Reyes *et al.* (2007), while lower incidence for the rice with milk was recorded by Hassan and Afifi (2016); Mohamed *et al*. (2016). Also, higher incidence for pudding (Hussein *et al*., 2015) and lower incidence for custard (Van Netton *et al*., 1990) were recorded. The different results obtained by researchers can be due to regional, seasonal, sampling and methodological differences. Also, the degree of contamination depends on the precautions observed during processing.

It is noticeable from Table 2 that most of the positive samples; 61.29% of the positive rice with milk samples, 68.18% of the positive pudding samples and 75% of the positive custard samples were in the range 10-<10^2 CFU/g. The obtained results are in agreement with that reported by Bryan *et al.* (1992) and Rosenquist *et al.* (2005).

The quality of the examined samples regarding the *B. cereus* count was estimated using the guidelines for the microbiological quality of ready-to-eat foods (NSW Food Authority, 2009), which shows four grades of the microbiological quality related to the *B. cereus* count; <10^2 CFU/g is considered good, 10^2-<10^3 CFU/g is considered acceptable, 10^3-<10^4 CFU/g is considered unsatisfactory and ≥10^4 CFU/g is considered unacceptable (potentially hazardous). It is clear from the results in Table 3 that 76% of the examined rice with milk samples, 92% of the examined pudding samples and 98% of the examined custard samples were considered good. Furthermore, 20%, 6% and 2% of the examined rice with milk, pudding, and custard samples, respectively were considered acceptable and only 4% of the examined rice with milk samples and 2% of the examined pudding samples were considered unsatisfactory. While, none of the examined samples was considered unacceptable (potentially hazardous). At the detected levels, *B. cereus* is not considered injurious to health, however, the risk will increase proportional to the detected levels and the likelihood of subsequent growth.

Since milk is the main constituent of the examined samples, it seems to be one of the sources of the contamination with the *B. cereus.* Soiling of cows' udders is one of the main sources of contamination of milk with *B. cereus*. The control of *B. cereus* in the dairy industry is challenging owing to the numerous points at which milk could get contaminated, either during production or processing. The number of *B. cereus* or its spores may be limited in raw milk by proper cleaning of the udder and teats before milking. It is worth mentioning that pasteurization kills the vegetative bacteria, but the spores survive. Pasteurization might even activate some of the spores (heat activation), which might start germinating (Tewari and Abdullah, 2015). In general, the food production facilities must use heating methods that destroy *B. cereus* vegetative cells and most spores. Also, cooked food should not be stored at room temperature (Montanhini *et al.*, 2013). While, cold storage may be a suitable control measure for such products. However, up to 14% of *B. cereus* strains may be psychrotrophic (Granum, 1997), and so temperature alone is not a practical control measure. Milk-based desserts should be prepared in small batches, cooled rapidly, and stored at 4°C (Smith *et al*., 2003).

In conclusion, the examined rice with milk, pudding and custard samples were contaminated with *B. cereus* in different percentages but in low numbers. However, rice with milk was more contaminated than pudding and custard. The current *B. cereus* load is not threatening to health but further growth of the bacteria at the retail premises until selling is likely leading to health hazards. Therefore, it is recommended to adopt measures to minimize the contamination with *B. cereus* from the very outset, even before processing in order to avoid the growth of the organism to dangerous levels. Also, better hygienic practices are required in the production of such products.

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تواجد الباسيلس سيريس فى بعض الحلاوي لبنية الأساس

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تم جمع ١٥٠ عينة من الحلاوي لبنية الأساس؛ الأرز باللبن، والمهلبية، والكسترد من مناطق مختلفة في مدينة أسيوط، مصر. وتم فحص العينات التي تم جمعها لعزل وعد الباسيلس سيريس. حيث تم استخدام تقنية MPN لعد الباسيلس سيريس باستخدام tryptone soy polymyxin broth ومستنبت MYP. وقد وجدت الباسيلس سيريس في ٦٢٪، ٤٤٪ و ٣٢٪ من عينات الأرز باللبن ، والمهلبية، والكسترد على التوالي. وكان عدد الباسيلس سيريس في أغلب العينات التي تم فحصها في نطاق 10^2 CFU/g>-10. وقد تمت مناقشة خطورة تواجد الباسيلس سيريس.

الكلمات المفتاحية: الباسيلس سيريس، الأرز باللبن، المهلبية، الكسترد.